mOsm/Kg H_2O thereby creating a condition suitable for retaining the shape and integrity of the leucocytes in the hematological sample.

13. The method according to claim 4 wherein the osmolarity of the leukocytes is about 400 mOsm/Kg.H_2O to about 600 mOsm/Kg.H_2O.

REMARKS

The Examiner rejected claims 4 and 13 under 35 U.S.C. §112, second paragraph, as being indefinite. To advance prosecution, Applicants have amended the claims in accordance with the Examiner's suggestions. Reconsideration and withdrawal of the rejection is therefore respectfully solicited.

As to claim 4, Applicants have adopted the suggested language provided by the Examiner in the Office Action. Support for the amendment appears in the specification at the paragraph bridging pages 11 and 12, and at page 12, lines 7-10. It respectfully is submitted that the amendment to claim 4 does not introduce new matter and entry and approval of the same, respectfully, is solicited.

With respect to claim 13, Applicants have amended the claim to better describe the osmolarity range indicated. Support for this amendment appears in the specification at page 12, lines 7-10. It respectfully is submitted that the amendment to claim 13 does not introduce new matter and entry and approval of the same, respectfully, is solicited.

Claims 1-13 were rejected under 35 U.S.C. §103(a) as being unpatentable over Loken et al., U.S. Patent No. 5,047,321 ("Loken") in view of Kim et al, U.S. Patent No.

5,559,037 ("Kim"), and Inami et al., U.S. Patent No. 5,624,663 ("Inami"). (Paper no. 12, p. 3). For the reasons presented below, reconsideration and withdrawal of the rejection respectfully is solicited.

Loken discloses a method for multi-parameter analysis of cells in a body fluid sample comprising two nucleic acid dyes and at least one fluorescently labelled cell surface marker (abstract). Loken further discloses analyzing said sample in an automated instrument capable of detecting and recording fluorescence of individual cells (col. 4, lines 37-40).

Kim discloses a method for the simultaneous and quantitative flow cytometric analysis of nucleated red blood cells and white blood cells in a whole blood sample comprising lysing red blood cells from an aliquot of the sample with a diluent to expose the red blood cell cytoplasm to a vital nuclear stain while inhibiting permeation of the stain into the white blood cells (abstract). Kim further discloses passing the diluent/sample mixture through an illuminated optical flow cell causing cells to scatter light and stained nuclei to fluoresce, said scattered and fluorescent light signals are then detected (col. 5, lines 3-10).

Inami, discloses a method comprising using a specific dye taken up by erythrocytic nucleated cells so that their nuclei are stained and differetiated by a flow cytometer (abstract). Inami further discloses a two step staining method using a first fluid that is a hypotonic solution comprising a fluorescent dye and a second fluid that is a solution that changes the osmorality and pH of the first fluid (col. 1, lines 56-59).

So that the record is clear, we summarize the rejections and the Examiner's arguments advanced against claims 1-13 as they presently stand.

In making the instant rejection, the Examiner relied on Loken for "teaching" a method comprising "combining a body fluid sample such as whole blood with at least two

nucleotide fluorescent dyes such as RNA dye or DNA dye and at least one fluorescent labeled antibody or cell surface marker to form a labeled mixture." and that the labeled mixture "is measured and analyzed using flow cytometric measurements of fluorescence intensity and light scatter for each cell examined." (Paper no. 10, at p. 6).

The Examiner acknowledged, however, that Loken did not disclose "increasing permeability of cytoplasm of specific nucleated cells, specifically erythroblasts using materials such as those in claim 4 of the instant invention, prior to incorporating RNA or DNA dyes thereto." (Paper no. 10, at p. 7).

To fill the acknowledged gap, the Examiner relied on Kim for "teaching" "mixing an aliquot of the blood sample with diluent which rapidly destroys the cytoplasm (lyses) of erythroblasts and erythrocytes and allowing exposure of erythroblastic nuclei while preserving the integrity and shape of the cytoplasm of leucocytes" which, the Examiner asserted, "have outstanding...qualities." (Id.).

To also fill the acknowledged gap, the Examiner relied on Inami for "teaching" "mixing blood with a hypotonic fluorescent dye solution capable of diffusing into erythroblasts to stain their nuclei and a buffer for maintaining pH in the acidic range." (Id. paragraph bridging pages 7 and 8).

The Examiner then contended that it would have been obvious to combine the teachings of Kim or Inami with the method of Loken, because, according to the Examiner, "Loken specifically expressed the need to analyze, discriminate, and count various populations of cell types which is not merely limited to leucocytes and Kim, likewise, expressed such a need including specifically counting and differentiating erythroblast populations in blood and bone marrow and Inami specifically suggested incorporating different dye elements into his method in

order to allow differentation of both erythroblasts and leucocytes." and because "it allows for simultaneous and accurate differentation and counting of both erythroblasts and leucocytes in a whole blood or bone marrow sample." and because it affords "reduced analytical time and accuracy in discriminating and counting of erythroblasts and leucocytes which are specific diagnostic indicators of diseases such as anemia and leukemia." (Paper no. 10, at p. 9).

In Paper no. 12, however, the Examiner contended that it would have been obvious to combine the teachings of Kim or Inami with the method of Loken because, "all three cited references utilize flow cytometry in differentiating stained/labeled nucleated cellular populations and Inami specifically suggested using his method in combination with specific nuclear dyes in order to allow better differentiation between erythroblasts and leucocytes," (paper no. 12, at p. 7) and because, "it allows for simultaneous differentiation between desired populations, in this case, erythroblasts from leucocyte populations." (Id.). The Examiner stated no other contentions supporting the rejection.

The Examiner provides no explanation or rational why her stated grounds for the necessary motivation or suggestion to support the rejection have shifted over the course of the two office actions. This is, however, not the only instance where the Examiner's position is unclear as to the specific nature and basis of a §103 rejection in this application.

The Examiner had previously rejected Applicants' claims under §103 over other combinations of the same references now cited in the instant rejection. In Paper no. 2, the Examiner issued two separate rejections under §103, one over Kim in view of Loken and the other over Inami in view of Loken. The Examiner withdrew these rejections and acknowledged in Paper no. 10, that Applicants had overcome these rejections through is proffered arguments

and amendments. Yet, in the same paper Examiner then issued the instant rejection under §103, over Loken, which became the primary reference, in view of Kim and Inami.

In making the rejection in Paper No. 2 under §103 over Loken in view of Kim, the Examiner stated that "one of ordinarily skill in the art" would be motivated to incorporate the teachings of Loken into the technique of Kim because "it allows for reduced analysis time coupled with greater accuracy in discriminating different cell types." Paper No. 2, page 9, para. 5).

In the same paper, the Examiner supported the rejection under §103 over Loken in view of Inami by stating "one of ordinary skill in the art" would have been motivated to incorporate the teachings of Loken into the technique of Inami "because it allows for reduced analysis time coupled with greater accuracy in discriminating and counting of erythroblasts in peripheral blood which are specific diagnostic indicators of diseases such as anemia and leukemia." Paper No. 2, page 11, para. 6).

Thus, the reasoning provided for supporting the withdrawn prior rejections are nearly identical to the reasoning supporting the instant rejection as set forth by the Examiner in Paper No. 10. It appears, in the reordering of the references which occasioned the more recent rejection under §103 of Loken in view of Inami and Kim, nonetheless, the Examiner finds ***the same*** motivation and suggestion in the new primary reference. Applicants wonder how, if their arguments previously overcome these references, the ***same contentions*** can support a different combination of the same references. Moreover, Applicants wonder how to effectively respond to these contentions when, as in Paper No. 12, they suddenly change.

Applicant respectfully requests that the Examiner indicate specifically what, if anything, is the specific motivation or suggestion she is relying upon to combine the cited

references and what sources or passages in the references, or what other sources, she invokes in support. Applicant presumes the Examiner's most recent of the different "motivations" postulated is what the Examiner now relies upon to demonstrate the requisite motivation to deviate from Loken, i.e. "it allows for simultaneous differentiation between desired populations, in this case, erythroblasts from leucocyte populations" and that the Examiner's other proffered "motivations" have been dropped. If this is incorrect, Applicant requests the Examiner apprise Applicants of the specific motivation relied upon to continue and finalize the rejection.

The MPEP succinctly states, "The mere fact the references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." MPEP §2143.01 (2000 Ed., 2100-98) Citing, *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990) (emphasis original). In issuing and finalizing the rejection, the Examiner only voices her opinion that the references can be combined, not any suggestion, motivation or disclosure to do so. A *prima facie* case under §103 requires far more than informed speculation.

With all due respect, the ***Examiner's*** motivation to combine the references is not relevant. Rather, the question is ***where*** in the cited references does this motivation to deviate from their teachings appear? *In re Rouffet*, 149 F.3d 1350, 47 USPQ2d 1453 (Fed. Cir. 1998) (Without a motivation in the references to combine them, the rejection held improper). Should the Examiner maintain the rejection, she is requested to explain "clearly and particularly" ***where*** the suggestion and motivation is found in the references of the rejection. *In re Dembiczak*, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999).

In fact, a review of Loken shows that the disclosed method calls for prefereable nucleotide fluorescent dyes, i.e. Thiazole-Orange and LDS-751. Other dyes usable are identified

in Loken as disclosed in U.S. Patent No. 4,544,546 (Loken, col. 4, lines 53-54). The dyes identified in Loken and in the referenced U.S. Patent include those that can penetrate the cell membrane without pre-treatment (see U.S. Patent No. 4,544,546, col. 5, lines 27 to 32). Given this, it undermines the Examiner's contention that the disclosure in Loken suggests or motivates combination of the method disclosed with that of Kim or Inami. To the degree Loken suggests or motivates use of dyes which would not utilize pre-treatment, Loken, in fact, teaches away from Kim or Inami.

Nor is there any factual support in Loken that supports the Examiner's contention that a combination of the method disclosed, with its particular dyes and treatments, would be compatible with the respective dyes and methods disclosed in Kim or Inami, however this combination may be effected absent undue experimentation. The absence of factual support, let alone the existence of factual grounds which contradict the Examiner's contentions, requires withdrawal of the rejection.

Moreover, the requisite suggestion and motivation cannot be supplied by mere statements as to the Examiner's belief that the requisite motivation is within "the level of skill in the art." *Al-Site Corp. v. VSI Int'l Inc.*, 50 USPQ2d 1161 (Fed. Cir. 1999). The Examiner must adduce support for this statement or it is insufficient for a *prima facie* case under §103. *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988). Merely noting advantages that could occur by the combination to one of skill in the art does ***not*** release the Examiner of her burden of demonstrating ***where*** in the references there appear any motivation to combine them and explaining ***why*** one would have chosen them.

Moreover, the Examiner must demonstrate the kind of motivation which would have "***strongly motivated***" one to pick the particular catalyst and temperature limitations required

by the claim, and to make a process as claimed [*Ex parte Graselli*, 231 USPQ 393, 394 (Bd. App. 1983)]. The type of motivation which would have "***impelled***" one to do so [*Levengood*, 28 USPQ2d at 1302], and the type of suggestion that the selection and combination "***should***" be made [*Ex parte Markowitz*, 143 USPQ 303, 305 (Bd. App. 1964)]. But that is what a conclusion of obviousness requires. See, *Levengood*, 28 USPQ2d at 1302. The Examiner has not addressed these elements, and without these elements, obviousness cannot be established. Opining as to *"motivations"* and *"suggestions"* falls far short of the type of ***factual evidence*** that § 103 demands.

In comparison to these requirements, the Examiner's stated rationale for the rejection is insufficient. By stating "It allows," the Examiner implies that the combination would "allow" one to arrive at the Applicants claims, i.e. if one was somehow otherwise motivated to do so. This not only fails to provide the requisite teaching or suggestion, it clearly applies an impermissible *per se* rule of obviousness, i.e. that the references are to be considered in light of the objectives achieved by the Applicants' invention. The teaching or suggestion to make the claimed combination must be found in the prior art, not the applicants' disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

Furthermore, by implying that the specific differentiation "in this case" is generally covered in the cited references, the Examiner admits the cited references do not specifically cover Applicants particular differentiation as claimed. Yet, the cited references must account for all claim elements and limitations. *In re Royka*, 180 USPQ 580 (CCPA 1974). Contending that they do merely because the references each deal generally with dyes and flow cytometry is not sufficient and does not account for the specific limitations and elements of the Applicant's claims; rather it only suffices to argue the cited references are analogous art, i.e.,

they are in the same general field of endeavor, not that they render the Applicants claims obvious. If by this contention the Examiner means to imply that any disclosure as to dyes and cytometry renders obvious all other methods involving these features, this is clearly wrong. MPEP §2141.02 (7th Ed., at 2100-94) (Distilling an invention down to the "gist" or "thrust" improper). If the cited references do not, as "in this case" they do not, disclose the differentiation of erythroblasts from leucocyte populations according to the method claimed, then all elements and limitations of Applicants claims have not been taught or suggested by the prior art, and the rejection must "in this case" fail.

In fact, Loken discloses use of two stains but fails to disclose discrimination of erythroblasts from leucocytes using the two dimensional scattergram. Inami discloses differentiating erythroblasts but utlizes only one dye and a second fluid containing buffers and an osmolarity adjustment agent. Kim discloses lysing red blood cells with a diluent in mixture with only one stain. Inami thus teaches away from use of both a binding antibody and a fluorescent dye. Kim teaches away from use of more than one stain (see, col. 5, line 35). In either case, each teach away from Loken's use of two dyes with no lysing agent.

Implying that all references share the same motivation because each wishes to enhance "diagnostic indicators of such diseases as anemia and leukemia" confuses an intent unspecified in the references with factual disclosure. It is well settled that an obviousness rejection must be based on facts, not generalities. *Ex parte Saceman*, 27 USPQ2d 1472, 1474 (BPAI 1993). "Cold hard facts." *In re Freed*, 165, USPQ 570, 571-72 (CCPA 1970). When a rejection under § 103 is not based on facts, it cannot stand. *Ex parte Porter*, 25 USPQ2d 1144, 1147 (BPAI 1992).

This is a fundamental failing in the rejection and one which the Examiner has yet to properly address. Generalities, speculation and noble pronouncements as to objectives do not replace a factual record; rather, these, only indicate a *per se* finding of obviousness. As also is well settled, there are no *per se* rules of obviousness. *In re Ochiai*, 37 USPQ2d 1127, 1133 (Fed. Cir. 1995) ("reliance on *per se* rules of obviousness is legally incorrect and must cease."); and see MPEP § 2116.01 at 2100-45 (Seventh Edition, Rev. 1, Feb. 2000). But a *per se* rule of obviousness, i.e., that if the disclosure of the cited references "allow" the Examiner to reach, "in this case," Applicants' claim limitations, then it is within the scope of the "prior art" and the claim is obvious, is precisely what the Examiner has employed here.

In sum, the Examiner has not adduced factual support demonstrating the requisite motivation for the combination of references advanced against applicants claims. The references, in whatever combination the Examiner may present, fail to account for all the Applicants claim limitations and, in fact, teach away from Applicants claims. The Examiner has not met her burden of demonstrating a *prima facie* case of obviousness, therefore, the rejection should be withdrawn.

Lastly, Applicants cannot let stand the Examiner's contention that Applicants merely attacked each individual reference, rather than their combination, in their prior response. For one, in view of the prosecution history and the Examiner's reordering of the cited references, Applicants are unsure what new combination of the same references they may face. Moreover, by demonstrating that each reference lacks teaching, suggestion or motivation for combination with the other cited references, Applicants showed the Examiner failed to make a *prima facie* case of obviousness. In this regard, Applicants conclusion that the references do not support the use of the dyes and reagents disclosed in a single method is a fact which remains

unconvtroverted by the Examiner. In contrast, mere speculation of what a combination will potentially "allow" fails to meet the applicable standard under §103.

In view of the foregoing, favorable action on the merits, and allowance of all claims, respectfully is solicited.

Respectfully submitted,

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April 17, 2001
Date of Signature

MARKED-UP CLAIMS

SERIAL NO. 09/058,323

4. (Thrice Amended) The method according to claim 1, wherein the raising of the permeability of the cell membranes of erythroblasts in the hematological sample to the nucleotide fluorescent dye in step (ii) comprises the steps of:

(i) admixing a first reagent fluid of hypotonic osmolarity containing a buffer for maintaining pH within an acidic range to the hematologic sample after [the] step (i) thereby raising the permeability of the erythroblast cell membranes to the nucleotide fluorescent dye; and

(ii) admixing thereto a second reagent fluid containing a buffer for neutralizing the first reagent fluid in the hematologic sample mixture and adjusting the pH of the mixture to an alkaline range of 5.0-11.0, thereby creating a condition suitable for staining the nuclei of the erythroblasts [maintaining a pH from 5.0 to 11.0] and an osmolarity compensating agent for adjusting the [an] osmolarity to about [from] 300 to 1000 mOsm/Kg H_2O thereby creating a condition suitable for retaining the shape and integrity of the leucocytes in the hematological sample.

13. (Amended) The method according to claim 4 wherein the osmolarity [integrity] of the leukocytes is about 400 mOsm/Kg.H_2O to about 600 mOsm/Kg.H_2O.